A diffusion tensor magnetic resonance imaging study of paediatric patients with severe non-traumatic brain injury

ERIKA MOLTENI[1] | MARIA A ROCCA[2] | SANDRA STRAZZER[1] | ELISABETTA PAGANI[2] | KATIA COLOMBO[1] | FILIPPO ARRIGONI[1] | GIACOMO BOFFA[2] | MASSIMILIANO COPETTI[3] | VALENTINA PASTORE[1] | MASSIMO FILIPPI[2]

1 Acquired Brain Injury Unit – Scientific Institute IRCCS Eugenio Medea, Bosisio Parini (LC); **2** Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan; **3** Unit of Biostatistics, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo (FG), Italy.

Correspondence to Massimo Filippi at Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy. E-mail: filippi.massimo@hsr.it

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ABBREVIATIONS

DRS	Disability Rating Scale
DT-MRI	Diffusion tensor magnetic resonance imaging
GOS	Glasgow Outcome Score
ICP	Inferior cerebellar peduncle
LOCFAS	Level of Cognitive Functional Assessment Scale
MCP	Middle cerebellar peduncle
MCS	Minimally conscious state
ROI	Region of interest
SCP	Superior cerebellar peduncle
VS/UWS	Vegetative state/unresponsive wakefulness syndrome

AIM In this observational study using 3T magnetic resonance imaging (MRI) and diffusion tensor, we investigated the differential effects of pathology, stage of disease, state of consciousness, and aetiology on the modifications of supra- and infra-tentorial white matter tracts and their correlations with clinical scales in paediatric patients with severe non-traumatic brain injury.
METHOD Diffusion tensor magnetic resonance imaging (DT-MRI) was obtained from seven children with unresponsive wakefulness syndrome (UWS; five males, two females; age at event 5y; standard deviation [SD] 2y 1mo), six children in a minimally conscious state (MCS; three males, three females; age at event 5y 10mo; SD 5y), and 10 healthy children as controls (two males, eight females; age at study 10y 10mo; SD 2y 10mo). Fractional anisotropy, mean, axial, and radial diffusivities were calculated for the corpus callosum, inferior, middle (MCP), and superior cerebellar peduncles (SCP).
RESULTS DT-MRI parameters from corpus callosum and SCP differed between patients and controls. MCP abnormalities were detected in patients presenting non-traumatic composite aetiology (n=4) versus those suffering from pure anoxia (n=9). The supra-tentorial compartment was more damaged (i.e. decreased fractional anisotropy and increased diffusivities) than the infra-tentorial one. Correlations were found between DT-MRI abnormalities and Glasgow Outcome Scale scores.
INTERPRETATION In paediatric UWS/MCS, the severity of clinical disability correlates with white matter tract abnormalities.

Hypoxic-ischaemic insult in the brain presents with variable patterns of damage to the neocortex, cerebellum, thalami, and brainstem,[1] involving supra- and infra-tentorial compartments.[2] It is characterized by a progression of pathophysiological responses, including cell swelling, free radical injury, and Wallerian degeneration, which are often superimposed to concurrent haemorrhagic or infectious damage, adding complexity to the final radiological picture. The most severe manifestations of hypoxic-ischaemic brain injury are the vegetative state/unresponsive wakefulness syndrome (VS/UWS) and the minimally conscious state (MCS). While brainstem lesions are equally common in these two states, the incidence of diffuse axonal injury and thalamic damage is higher in the VS/UWS.[3,4] This suggests that severe disorders of consciousness are disconnection syndromes involving both the brainstem and long-range thalamo–cortical and cortico–cortical pathways.[3,4] At birth, myelination is incomplete.[5] Depending on age at insult, hypoxic-ischaemic damage during childhood acts as a conditioning factor on axonal myelination, fibre organization and brain maturation,[6] with detrimental effects on cognitive and motor development and on the acquisition of adequate perception of self and of the environment. Cognitive assessment plays a key role in the management and outcome prediction of patients with disorders of consciousness.[7] However, single clinical scales offer limited sensitivity to the subtle changes that may occur in these patients,[8] as they can only probe a limited set of cognitive functions. As a consequence, the administration of multiple scales, covering wider spans of responses, is usually preferred.

Diffusion tensor (DT)-MRI is used to inspect the anisotropic physical properties of the white matter and reconstruct fibre orientation. White matter damage often results in decreased fractional anisotropy,[9–11] and increased axial, radial, and mean diffusivities.[11]

In this study, we applied DT-MRI to quantify damage in several supra- and infra-tentorial white matter tracts in paediatric patients with hypoxic-ischaemic VS/UWS and MCS in comparison with age-matched healthy controls with the goals of investigating possible differential effects of pathology, stage of disease, state of consciousness, and aetiology on the modifications of tract organization. To explore the clinical relevance of DT-MRI findings, we assessed their correlations with clinical and cognitive measures of patients' impairment.[12,13]

What this paper adds

- In paediatric non-traumatic brain injury, fractional anisotropy decreases and diffusivities increase.
- The supra-tentorial compartment is more affected than the infra-tentorial one.
- Fractional anisotropy and diffusivities abnormalities correlate with Glasgow Outcome Scale.
- Global, rather than focal, damage contributes to clinical severity.

PARTICIPANTS AND METHODS

Participants

Fifteen paediatric patients (10 males, five females; age at injury 6y 6mo; standard deviation [SD] 4y 7mo) were recruited from the Scientific Institute 'E. Medea', Bosisio Parini, Italy. Patients were eligible for the research if: (1) they had a diagnosis of acquired brain injury, based on anamnestic, clinical, and neuroimaging data; (2) the acquired brain injury had a non-traumatic origin; (3) they had the diagnosis of UWS/VS or MCS, according to the diagnostic criteria of the Multi-Society Task Force[14] and the Royal College of Physicians;[15] (4) age at insult was between 2 (to guarantee that myelination has mainly completed) and 16 years (to confine the study to the paediatric period);[16] and (5) they were right handed (if of school age).

The exclusion criteria were (1) congenital neurological pathology, and (2) inability to undergo MRI (e.g. metal implants or claustrophobia). Two patients were subsequently excluded from the analysis because of artefacts invalidating the MRI. The final cohort resulted in 13 paediatric patients (eight males, five females; age at injury 5y 5mo; SD 3y 7mo).

Ten age- and sex-matched, right-handed paediatric healthy volunteers (two males, eight females; mean age 10y 11mo; SD 2y 10mo; range 7y 5mo–17y 8mo) with no previous history of neurological dysfunction, and with a normal neurological examination, were recruited as controls.

Approval was received from the ethical committee of the Scientific Institute 'E. Medea' (Bosisio Parini, Italy) and written informed consent was obtained from all the parents/guardians of the children participating in the study. The study was conducted in compliance with the Declaration of Helsinki.

Clinical evaluation

The patients' medical histories, including the Glasgow Coma Scale (GCS) score at insult and the days of coma, were collected. Neurological evaluation was performed by experienced personnel, unaware of MRI results, and included: (1) the Glasgow Outcome Score (GOS);[17] to objectively assess the degree of recovery; (2) the Disability Rating Scale (DRS); (3) the Level of Cognitive Functional Assessment Scale (LOCFAS),[12] for the evaluation of the cognitive functions on the basis of directly observable behavioural manifestations; and (4) the Coma/Near Coma Scale (C/NCS),[13] to estimate the depth of coma. High scores on the GCS, LOCFAS, and GOS reflect less severe clinical impairment, whereas high scores on the DRS and C/NCS indicate more severe impairment.

White matter tract analysis

The protocol for MRI acquisition and analysis is described in Appendix S1 (online supporting information). At the first stage of the analysis, we attempted to track a comprehensive set of brain white matter tracts using DT tractography, as illustrated in Catani et al.[18] The set included a number of supra-tentorial long-range associative and projection tracts such as the corpus callosum, cingulum and inferior fronto-occipital fasciculus, etc. However, the severity of brain damage affecting our patient cohort meant that only the cerebellar peduncles could be reconstructed reliably. As a consequence, to have a measure of the extent of supra-tentorial damage, we studied the corpus callosum, applying a region of interest (ROI) approach.

As a general rule for delineating the ROIs to initialize tractography propagation, we combined the information provided by both fractional anisotropy and colour coded brain maps. ROI selection and tract propagation methods applied to the cerebellar peduncles are detailed in Appendix S1. Each tract was then exported as an image. The portion of tracts exceeding previously defined limits were removed using the FslView program (http://fsl.fmrib.ox.ac.uk/fsldownloads/; FSL, Oxford, UK) (Fig. 1a). To minimize partial volume contamination from cerebrospinal fluid, we confined the analysis to the centre of the tract. To do this, the fractional anisotropy map from each subject was skeletonized using FSL software and the fractional anisotropy skeletonized map was then applied to mask the images of the tracts (Fig. 1b). Skeletonized tracts of inferior cerebellar peduncle (ICP), middle cerebellar peduncle (MCP), and superior cerebellar peduncle (SCP) entered the statistical analysis.

The corpus callosum was initially selected on the midsagittal slice by placing a single ROI, which was then subdivided into five parts, according to a previously described geometric method.[19] The method is based on computation of five equal sections on the straight line connecting the anterior commissure and posterior commissure points. From the edges of the selected sections, vertical lines were drawn, intersecting the corpus callosum ROI on the ventral boundary. Then, radial lines were drawn, passing through the midpoint of the anterior commissure-posterior commissure line and the points of intersect on the ventral boundary of the corpus callosum, up to the dorsal boundary of the corpus callosum. As a result, a five-region

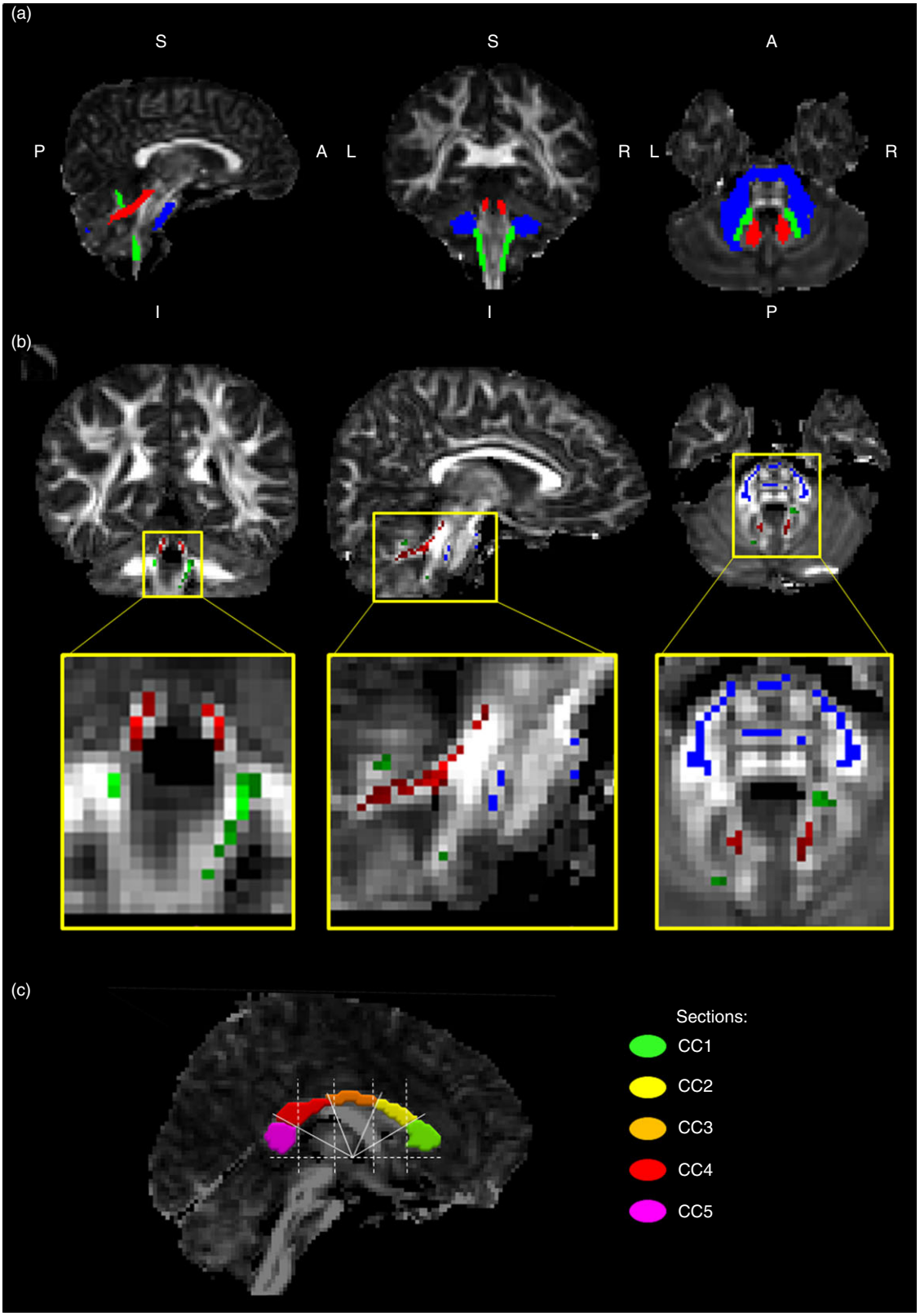

Figure 1: (a) Example of diffusion tensor tractography reconstruction of the cerebellar peduncles in a control participant. The inferior cerebellar peduncle is shown in green, the middle cerebellar peduncle is in blue, and the superior cerebellar peduncle is in red. (b) Results of skeletonization of the previous peduncle tracts. Left is left. (c) Corpus callosum (CC) extraction, by application of a region of interest (ROI) approach: five sections are partitioned along the frontal-occipital span. [Colour figure can be viewed at wileyonlinelibrary.com].

partition of the ROI was obtained. The five regions were numbered CC1 to CC5, moving from the frontal towards the occipital part of the corpus callosum (Fig. 1c).

Statistical analysis

Normal distribution assumption on residuals of each model was checked by means of a Q–Q plot and Shapiro–Wilks and Kolmogorov–Smirnov tests. Between-group comparisons were performed using ANCOVA models, adjusted for age. The following effects on DT-MRI measures were evaluated: (1) pathology (patients vs controls); (2) stage of disease (acute/subacute vs chronic condition); (3) state of consciousness (VS/UWS vs MCS); and (4) aetiology (pure anoxic vs composite hypoxic-ischaemic brain damage).

Acute/subacute condition was defined for a period <12 months from the anoxic event, while the chronic stage of disease was defined for a time interval ≥12 months. Pure anoxia was defined as caused by suffocation, drowning, or embolism, while composite anoxia was ascribed to all the other cases (e.g. infection, encephalopathy).

DT-MRI measures were tested for difference in laterality (left vs right) using paired Student's *t*-tests. Because no statistically significant laterality effects were detected, the mean values of left and right tracts were used in the analysis. DT-MRI measures derived from infra- and supra-tentorial compartments were compared between patients and controls using fixed-effect hierarchical linear models, accounting for the repeated measures design. In patients, age-adjusted correlations between DT-MRI measures and clinical scales were assessed using Spearman's rank correlation coefficient. All analyses were adjusted for multiple comparisons using the false discovery rate approach (false discovery rate <0.2 was considered to be statistically significant).

All analyses were performed using SAS Release 9.3 (SAS Institute, Cary, NC, USA) statistical software.

RESULTS

Clinical data

Table I summarizes the main demographic and clinical features of patients. The group comprised eight males and five females, their mean age at insult was 5 years and 5 months (range 2y 4mo–15y 8mo), and their mean age at study was 6 years and 11 months (range 2y 10mo–16y). The aetiology was encephalopathy in four patients (31%), drowning in five (38%), suffocation in three (23%), and embolism in one (8%). At the time of MRI acquisition, nine (69%) patients were in the acute/subacute stage and four (31%) in the chronic condition. Seven patients (54%) were in a UWS/VS, while six patients (46%) were in an MCS. The mean number of days of coma for the patients in an MCS was 91 (SD 50).

MRI analysis

Difference in head motion and eddy current effects during the scan did not differ between patients (max linear displacement was 11.4 [SD 4.8]; max linear rotation was 0.08 [SD 0.13]) and controls (max linear displacement was 14.8 [SD 8.7]; max linear rotation was 0.05 [SD 0.03]). The results of anatomical examination for each participant are reported in Table SI (online supporting information). DT-MRI measures in patients and controls passed the test for normal distribution assumption and are shown in Table II and Table SII (online supporting information). Compared with controls, patients had statistically significant reduced fractional anisotropy and increased mean diffusivity in the total corpus callosum, the five corpus callosum sections separately, and the SCP. Compared with controls, patients had increased axial diffusivity in the total corpus callosum, the different corpus callosum sections (except for the occipital one), SCP, and total MCP. Finally, radial diffusivity was increased in patients compared with controls in the total corpus callosum, the five corpus callosum sections, and SCP. DT-MRI measures derived from the anterior portion of the MCP did not statistically differ between patients and controls (data not shown).

Compared with patients suffering from pure anoxia (n=9), patients presenting non-traumatic composite aetiology (encephalopathy) (n=4) had increased radial diffusivity in the total MCP (Table II).

DT-MRI measures did not differ between UWS/VS (n=7) and MCS (n=6) patients, nor between patients in the acute/subacute (n=9) stage of disease and those in the chronic stage (n=4).

The differences in fractional anisotropy and radial diffusivity between supra- and infra-tentorial compartments were different between patients and controls (Table III). The difference in fractional anisotropy between supra- and infra-tentorial compartments was different between UWS/VS and MCS patients (p=0.04). No differences were found between acute/subacute and chronic patients as well as patients with anoxia versus those with composite aetiology.

Correlations between DT MRI measures and clinical scales

GOS correlated with fractional anisotropy in the different corpus callosum sections (except for the parietal one) and the total corpus callosum (maximum r was 0.73, with false discovery rate=0.04) (Table SIII, online supporting information). GOS also correlated with radial diffusivity in corpus callosum sections (CC1, CC2, and CC5) and the total corpus callosum (maximum r was −0.69, with false discovery rate=0.12) (Table SIII).

No correlations were found between DT-MRI indexes and LOCFAS, C/NCS, and DRS clinical scales.

DISCUSSION

VS/UWS and MCS are clinical presentations of a functional cortical disconnection syndrome. Previous positron emission tomography studies of VS/UWS have observed 'functional disconnections' between lateral prefrontal and midline posterior cortices and between nonspecific thalamic nuclei and midline posterior cortices,[20] which restored near normative metabolic values after recovery of consciousness.[3] The underlying pattern of grey matter

Table I: Main demographic and clinical characteristics of the patients in the study

Number	Sex	Age at event	Age at study	Stage of disease	Aetiology	UWS/VS or MCS	Days of coma[a]	Glasgow Coma Scale	Glasgow Outcome Score	Disability Rating Scale	Level of Cognitive Functional Assessment Scale	Coma/Near-Coma Scale
1	M	5y 5mo	5y 7mo	Acute/Subacute	Suffocation	UWS/VS	>365	4	2	23	2	2
2	M	4y 4mo	6y	Chronic	Encephalopathy	UWS/VS	>365	4	2	23	3	2
3	F	15y 8mo	16y	Chronic	Embolism	MCS	130	3	2	22	3	2
4	M	4y	13y 5mo	Chronic	Drowning	UWS/VS	>365	4	2	23	2	3
5	F	5y 6mo	5y 10mo	Acute/Subacute	Encephalopathy	MCS	160	4	3	21	3	2
6	M	2y 7mo	2y 10mo	Acute/Subacute	Encephalopathy	MCS	90	3	2	23	n.a.	2
7	F	6y 2mo	6y 6mo	Acute/Subacute	Drowning	MCS	90	3	3	12	6	1
8	F	6y 11mo	7y 8mo	Acute/Subacute	Encephalopathy	UWS/VS	>365	6	2	22	2	2
9	M	2y 11mo	3y 1mo	Acute/Subacute	Drowning	MCS	25	3	3	20	n.a.	1
10	M	8y 7mo	8y 11mo	Acute/Subacute	Drowning	UWS/VS	>365	3	2	23	3	2
11	M	2y 7mo	2y 11mo	Acute/Subacute	Drowning	MCS	50	3	3	22	n.a.	1
12	F	3y 7mo	6y 7mo	Chronic	Suffocation	UWS/VS	>365	3	2	22	n.a.	2
13	M	2y 4mo	3y 1mo	Acute/Subacute	Suffocation	UWS/VS	>365	3	2	22	n.a.	2
Mean (SD)	8M/5F	5y 5mo (3y 7mo)	6y 11mo (4y)					3.5 (0.9)	2.3 (0.5)	21.4 (3.0)	3.0 (1.3)	1.8 (0.6)

n.a. indicates not assessable, because of age <4y. Stage of disease: acute/subacute<12mo, chronic≥12mo. [a]Days of coma >365 indicate that the patient remained in UWS/VS. UWS/VS, unresponsive wakeful syndrome/vegetative state; MCS, minimally conscious state.

Table II: DT-MRI measures from patients and healthy controls. Data from patients with pure anoxic aetiology and composite aetiology are also shown

Measure	Controls	Patients	*p*(FDR)	Anoxic patients	Composite patients	*p*(FDR)
Tot CC FA	**0.53 (0.04)**	**0.29 (0.10)**	**<0.001**	0.29 (0.08)	0.29 (0.14)	0.963
Tot CC MD	**1.04 (0.08)**	**1.56 (0.20)**	**<0.001**	1.54 (0.18)	1.61 (0.27)	0.679
Tot CC AD	**1.71 (0.09)**	**2.01 (0.15)**	**0.002**	1.99 (0.17)	2.06 (0.09)	0.562
Tot CC RD	**0.71 (0.09)**	**1.34 (0.26)**	**<0.001**	1.32 (0.22)	1.38 (0.37)	0.791
SCP FA	**0.47 (0.03)**	**0.40 (0.08)**	**0.050**	0.40 (0.08)	0.39 (0.10)	0.963
SCP MD	**2.41 (0.18)**	**4.18 (0.96)**	**<0.001**	4.00 (0.87)	4.59 (1.13)	0.504
SCP AD	**1.27 (0.10)**	**1.98 (0.34)**	**<0.001**	1.90 (0.34)	2.14 (0.34)	0.472
SCP RD	**0.57 (0.05)**	**1.10 (0.31)**	**<0.001**	1.05 (0.28)	1.23 (0.40)	0.611
Tot MCP FA	0.48 (0.04)	0.48 (0.06)	0.339	0.51 (0.05)	0.43 (0.02)	0.268
Tot MCP MD	2.13 (0.09)	2.30 (0.28)	0.340	2.18 (0.18)	2.56 (0.32)	0.323
Tot MCP AD	**1.13 (0.05)**	**1.22 (0.11)**	**0.096**	1.19 (0.09)	1.27 (0.13)	0.472
Tot MCP RD	0.50 (0.03)	0.54 (0.10)	0.693	**0.50 (0.06)**	**0.64 (0.09)**	**0.125**
ICP FA	0.49 (0.04)	0.47 (0.06)	0.890	0.48 (0.07)	0.44 (0.04)	0.963
ICP MD	2.14 (0.13)	2.20 (0.22)	0.743	2.15 (0.19)	2.31 (0.28)	0.528
ICP AD	1.14 (0.05)	1.13 (0.08)	0.899	1.12 (0.05)	1.16 (0.14)	0.562
ICP RD	0.50 (0.05)	0.53 (0.08)	0.693	0.51 (0.08)	0.57 (0.07)	0.611

Significant values are in bold. Measures are given as mean (SD). Mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) are expressed in units of $mm^2/s \times 10^{-3}$; fractional anisotropy (FA) is a dimensionless index. FDR, false discovery rate; Tot CC, total corpus callosum; SCP, superior cerebellar peduncle; Tot MCP, total middle cerebellar peduncle; ICP, inferior cerebellar peduncle.

Table III: Comparison of DT-MRI measures as mean (standard deviation) from the supra- and infra-tentorial districts between patients and controls

Measure	District	Controls	p^a	Patients	p^a	p^b
FA	Supra-tentorial	**0.51 (0.02)**	**0.081**	**0.27 (0.02)**	**<0.001**	**<0.001**
FA	Infra-tentorial	**0.48 (0.02)**		**0.45 (0.02)**		
MD	Supra-tentorial	1.03 (0.08)	**<0.001**	1.61 (0.07)	**<0.001**	0.465
MD	Infra-tentorial	2.21 (0.10)		2.91 (0.09)		
AD	Supra-tentorial	1.69 (0.04)	**<0.001**	2.03 (0.04)	**<0.001**	0.540
AD	Infra-tentorial	1.17 (0.05)		1.45 (0.05)		
RD	Supra-tentorial	**0.71 (0.05)**	**<0.001**	**1.39 (0.05)**	**<0.001**	**<0.001**
RD	Infra-tentorial	**0.51 (0.06)**		**0.74 (0.05)**		

Hierarchical linear models. Significant values are in bold. Measures are given as mean (SD). Mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) are expressed in units of $mm^2/s \times 10^{-3}$, fractional anisotropy (FA) is a dimensionless index. [a]Comparison between the supra- versus infra-tentorial compartments. [b]Supra- versus infra-tentorial differences between controls and patients.

structural damage most often encompasses the polymodal associative areas, with relative sparing of structures involved in arousal and autonomic functions, such as the brainstem pedunculopontine reticular formation, hypothalamus, and basal forebrain.[20]

Here, we analyzed modifications of physical properties of the brain white matter of paediatric patients with hypoxic-ischaemic insult. We applied stringent enrolment criteria, excluding those patients with a possible traumatic influence on the pattern of brain damage. We investigated brain white matter tracts encompassing both the infra- and supra-tentorial districts. Special attention was devoted to the cerebellar peduncles, as brainstem lesions are known to be equally common in VS/UWS and MCS but also to have a differential role in the two states. We studied the differential effects of pathology, stage of disease, state of consciousness, and aetiology on diffusivity abnormalities and their correlations with patients' clinical impairment.

In agreement with previous works showing reduced fractional anisotropy in the corpus callosum and brainstem in adults[10,11] and children[9,21] with these conditions, we found reduced fractional anisotropy and increased diffusivity in corpus callosum and SCP in our group of patients. The ICP did not differ between patients and controls, whereas the MCP had only increased axial diffusivity. These results are in line with the gradient of damage commonly observed in hypoxic-ischaemic patients, which prevalently affects the supra-tentorial district.[22] At the single patient level, great variability was observed in DT-MRI measures of MCP, which probably contributed to nullify possible statistical effects of pathology, with a subset of patients clearly showing no decrease of fractional anisotropy and no increase of diffusivity values. The MCP is the largest among the cerebellar peduncles. In the circumstance of a traumatic impact causing a cranial-caudal mechanical strain, the preferential preservation of MCP appears straightforward; indeed, MCP fibres show mainly posterior-anterior and latero-lateral course, in contrast to the chiefly cranial–caudal path of SCP and ICP. The preferential ICP and MCP preservation found in our non-traumatic cohort rules out the concussive hypothesis; rather, it supports an exclusive capability of MCP and ICP fibres to resist degeneration, at least in a subset of patients.

Because of the small number of patients, we detected no difference in DT-MRI measures between UWS/VS and MCS. A previous study found lower fractional anisotropy and higher radial diffusivity in several brain structures in patients with unfavourable versus favourable outcome;[10]

another study described decreased fractional anisotropy of the anterior pons and bilateral midbrain.[2] A direct comparison between previous and our results is inaccurate, however, because they grouped UWS/VS and MCS conditions in the unfavourable outcome group. Recently, Fernàndez-Espejo et al.[4] observed lower mean diffusivity values in patients in a vegetative state compared with those with MCS, and proposed such an index for the evaluation of the remaining brain healthy tissue.

The small size of our sample might have influenced the comparison between chronic and acute/subacute patients, which showed no differences. A previous work[11] observed that, compared with the acute stage, fractional anisotropy in the corpus callosum and corona radiata further decreased and radial diffusivity increased 2 years after the event.

Compared with patients with composite aetiologies, those with pure anoxia had less severe MCP damage which might hint towards an additive detrimental role of the plurality of lesions, with respect to single focal lesions.

Finally, the analysis of DT-MRI measures in the supra- and infra-tentorial districts revealed an abnormal ratio for fractional anisotropy in patients compared with controls, and showed differences in radial diffusivity. This is in line with the gradient of damage commonly ensued from the hypoxic-ischaemic event in patients, which prevalently affects the supra-tentorial district and causes inversion of the supra/infra-tentorial ratio for fractional anisotropy between patients and controls.[22]

Poor scores at GOS correlated with reduced fractional anisotropy in the posterior corpus callosum midbody, which hosts fibres connecting the primary motor areas. GOS scores also correlated with fractional anisotropy and radial diffusivity values in the rostrum, genu, and anterior midbody, which connects the prefrontal/orbitofrontal cortices and the premotor/supplementary motor areas, and in the splenium, which connects the parietal and temporal lobes and the visual cortex. These results generalize previous findings of positive correlations between higher fractional anisotropy and higher GOS scores in the posterior aspect of the corpus callosum[10] and in the total corpus callosum,[9] and further support GOS as the most informative clinical score in terms of neural correlates.

Importantly for rehabilitation field, LOCFAS, C/NCS, and DRS did not correlate with DT-MRI abnormalities. This could be partly because the LOCFAS is hardly applicable and provides unreliable results for children younger than 4 years, and consequently it was not administered to five patients in this study. Additionally, with the exception of one patient, all patients scored either 2 or 3, thus not favouring the attainment of a reliable correlation evaluation. DRS fathoms cognitive abilities and self-care activities which imply a too high functional level, with respect to the capabilities of our cohort.

A recent voxel-based meta-analysis of DT-MRI in 583 patients with mild traumatic brain injury identified three brain regions in which patients show lower fractional anisotropy values compared with healthy controls. The largest region comprises the thalamus and extends to the splenium of the corpus callosum, the second includes the forceps minor, and the third is located in the superior longitudinal fasciculus.[23] Patients in the present study showed a broader pattern of damage: fractional anisotropy decrease extended to the entire corpus callosum, and fractional anisotropy in the telencephalon was so low that the reconstruction of the tracts was unobtainable. Several factors might contribute to these findings, including: (1) we considered patients with hypoxic, rather than traumatic, brain damage; (2) brain injury was severe in all cases; and (3) fractional anisotropy in childhood can be sensibly lower than in adulthood. Single studies in children with traumatic brain injury testify that fractional anisotropy values are reduced in the genu of the corpus callosum, posterior limb of internal capsule, superior longitudinal fasciculus, superior fronto-occipital fasciculus, and centrum semiovale.[24,25] Additionally, the previous fractional anisotropy abnormalities correlated with GCS scores.[24,25]

Our results deserve also some methodological considerations. When enrolling paediatric cohorts, age range selection implicitly imposes conditions on brain development and brain maturation stages, exerting some bias on anatomo-functional measures. While designing the present DT-MRI paediatric study, we had to account for the possible role of incomplete myelination. We enrolled patients aged between 2 years and 16 years. Previous studies[16] observed an initial sharp increase of fractional anisotropy in cerebral white matter, up to the age of 24 months, followed by a more gradual increase up to 132 months; analogously, mean diffusivity showed a sharp decrease in cerebral white matter up to 24 months, followed by a moderate decrease thereafter. In cerebellar white matter, increased fractional anisotropy was observed up to 36 months, while decreased mean diffusivity lasted 6 months. Because we were interested in studying white matter tracts, we chose the age of 2 years as the lower limit for patient enrolment. Patient 3 was older than all remaining patients at the time of injury. However, we decided to keep her in the analysis because all her DT-MRI values were within the range obtained from the remaining patients' group (Fig. S1, online supporting information).

The presence of crossing fibres is a potential confounder in the results. MCP pathway reconstruction by DT-MRI is prone to artificial enlargement, because of the inclusion of collinear and crossing fibres not inherent to the pathway.[25] Specifically, the transverse fibres of the pons overlap the anterior MCP, which could explain the apparent partial preservation of the MCP in patients. Given that the tensors do not model crossing fibres, a lack of difference in fractional anisotropy and mean diffusivity of the MCP in patients compared with controls does not necessarily indicate a preservation of fibre tracts in patients. To control for this possible confounder, and to gain maximal standardization through participants, we confined the analysis to the centre of the tracts by defining anatomical landmarks specific to each tract, to limit the streamlines, and

we masked each tract with a skeletonized fractional anisotropy map.

Lastly, correlations between DT-MRI measures and GOS should be regarded with caution, as we only observed two GOS levels in our cohort. This is because of the homogeneity of the cohort itself, but poses limitations on the statistical value of the results, which are rather supportive of certain discriminative capabilities of the correlating DT-MRI measures.

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SUPPORTING INFORMATION

The following additional material may be found online:

Appendix S1: Protocol for MRI acquisition and analysis.

Table SI: Lesion description from the neuroradiological examination.

Table SII: DT-MRI measures as mean (SD) from the five corpus callosum sections of patients and healthy controls.

Table SIII: Correlations between the GOS scale and DT-MRI measures in patients.

Figure S1: DT-MRI derived metrics calculated for Patient 3 (pink line), with reference to those obtained for the whole patient group (black band) and listed in Table II.

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